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INFLUENCE OF SEED BACTERIZATION ON NODULATION, YIELD AND AMINO ACID COMPOSITION OF SEED PROTEIN OF *CICER ARIETINUM* VAR. TYPE-1

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ABSTRACT

The role of different strains of *Rhizobium* sp. (gram) H_{44}, H_{45}, *Beijerinckia indica* J_3 and *Azotobacter chroococcum* B_4, on nodulation, seed yield and amino acid compostion of seed protein of *Cicer arietinum* var. type-1 were assessed under the field conditions, following the randomized block design with a basal dressing of 20 kg N and 50 kg P_2O_5/ha. Simultaneous inoculation with *Azotobacter* and any of the strains of *Rhizobium* proved better than *Rhizobium* or *Azotobacter* or *Beijerinckia* or *Rhizobium*+*Bejerinckia* towards the yield, Out of the 16 amino acids detected in the seeds, aspartic acid, glutamic acid, isoleucine and phenylalanine were maximum due to strain H_{45}; threonine, glycine and valine due to strains $H_{45} + J_3$; tyrosine and histidine due to strains $H_{45} + B_4$; and alanine due to strain B_4. Aspartic and glutamic acids tended to decrease due to simultaneous inoculation with *Rhizobium* and *Azotobacter* when compared with *Rhizobium* or *Azotobacter* alone; whereas some of the amino acids showed an increasing trend. *Azotobacter* when used with *Rhizobium* caused the formation of fewer but healthier nodules and perhaps the better utilization of the symbiotically fixed nitrogen by the crop.

YIELD increases were reported by Sheloumova[8] (1941) with simultaneous treatment of some leguminous seeds with azotobacterin and root nodule bacteria specific for them. Synergistic effect of gram *Rhizobium* H_{44} and *Azotobacter chroococcum* B_4 was recorded by Rawat and Sanoria[6] (1976) on the grain yield. Employing *Rhizobium*, *Azotobacter* and *Beijerinckia* either alone or in combinations of the later two with *Rhizobium*, experiments were tried following the same design and technique. The primary objective was to assess the effect of inoculation on both the quality and quantity of seeds.

MATERIAL AND METHODS

Gram rhizobia, *Beijerinckia indica* and *Azotobacter chroococcum* used in this study are isolates from Sahapura block soils of Jabalpur, kitchen garden soil of Jabalpur and B.H.U. farm soil of Varanasi respectively. *Rhizobium* isolates were serologically distinct (Vaishya[9], 1971) while *Beijerinckia* and *Azotobacter* were morphologically different. *Beijerinckia* had the capability to grow at pH 3·0 and it produced abundant slime. *Rhizobium* was grown on yeast extract mannitol agar medium (Vincent[10], 1970) slants and *Azotobacter* and *Beijerinckia* were grown on Burk's medium (Rubenchik[7], 1963). Preparation of cultures and the method of seed inoculation was the same as described by Rawat and Sanoria[6] (1976).

The plants were uprooted for nodule study 70 days after sowing. Crude protein in seeds was determined by Kjeldahl method and the amino acids were estimated with Hitachi-Perkin Elmer KLB-3B automatic amino acid analyser.

RESULTS AND DISCUSSION

Nodule weight, instead of the nodule number, is considered to be a better criterion for evaluating the efficiency of rhizobia. Extent of symbiotic nitrogen fixation thus depends upon the nodular mass. Sometimes irregularities do appear because of the formation of nodules by inefficient but active strains. In such cases the nodules will either be deprived of or remain poor in leghemoglobin, the real compound of symbiotic origin (Burris[2], 1974). Looking to the data on nodulation, yield and crude protein content of the seeds (Table I), it appears that seed bacterization with either *Azotobacter* or *Beijerinckia*, stimulates slightly the native rhizobia which in turn causes somewhat better nodulation over the control. Both the strains of gram *Rhizobium* sp. brought about greater nodulation and increased the nodular mass but the grain yield was not significantly improved over the control. Of course, *Rh.* H_{44} promoted the vegetative growth. Similarly, *Rh.* H_{45} with *Beijerinckia* was beneficial for the straw yield. When *Rhizobium* strains were used along with *Azotobacter* or *Beijerinckia*, there was reduction in the nodule numbers in comparison to *Rhizobium* alone. In contrast to *Azotobacter*, *Beijerinckia* inoculation, along with *Rhizobium*, decreased the nodular mass.

From the point of view of crop yield, treatment *Rh.* H_{44} + *Azotobacter* stood first and next to it was the treatment, *Rh.* H_{45} + *Azotobacter*. Rao Kumar and Patil[5] (1976) reported better yields of soybean due to inoculation with *Rhizobium* and *Azotobacter* whereas Rajani Apte and Iswaran[4] (1974) obtained good yields of soybean with *Rhizobium* and *Beijerinckia*. By the use of *Azotobacter* with *Rh.* H_{44} or *Rh.* H_{45}, although there was reduction in the number of nodules yet, the weight of individual nodules had increased. This observation supports the hypothesis that the total nodule weight (instead of nodule number) should be considered as a criterion in legume *Rhizobium* symbiosis. In the treatment of *Rh.* H_{45} the weight of nodules/

TABLE I

Nodulation, characteristics, yield and crude protein content of Bengal gram

Treatments	Total No. of nodules per plant	Oven dry weight of nodules per plant	Straw yield (Q/ha)	Grain yield (Q/ha)	Seed crude protein (%)
1. Control	3·25	14·25	34·75	24·58	24·71
2. *Rhizobium* sp. H_{44}	56·75**	160·25**	45·37*	26·50	25·65
3. *Rhizobium* sp. H_{45}	31·00**	133·00**	41·50	25·50	24·99
4. *Beijerinckia indica* J_3	17·00	36·50	40·37	24·00	25·25
5. *Azotobacter chroococcum* B_4	11·00	27·50	40·50	24·58	24·26
6. *Rh.* sp. H_{44} + *Beij. indica* J_3	33·00**	96·50**	40·75	23·58	23·34
7. *Rh.* sp. H_{44} + *A. chroococcum* B_4	24·75**	202·25**	47·75**	29·50**	25·35
8. *Rh.* sp. H_{45} + *Beij. indica* J_3	23·25*	94·75*	46·37**	24·75	23·74
9. *Rh.* sp. H_{45} + *A. chroococcum* B_4	21·75*	104·00**	47·50**	28·25*	24·30
C.D. at 5%	17·95	60·26	8·15	3·11	N.S.
1%	24·38	81·67	11·04	4·17	N.S.

* Significant at 5% level of significance. ** Significant at 1% level of significance.

plant is 133 mg whereas in the treatment *Rh.* H_{45} + *Azotobacter* it is 104 mg. That the yield was increased by the latter treatment, and not in the former, warrants for an alternative explanation.

In the absence of data on leghemoglobin content which could have been taken as the parameter for the amount of nitrogen fixed symbiotically, one has to consider the mode of utilization of the fixed nitrogen. Referring to data in Table II on amino acid composition of seeds, some explanation is possible. In the control, the amino acids serine, proline, leucine, lysine and arginine are in the highest amount. *Rh.* H_{45} gave maximum amount of aspartic acid, glutamic acid, isoleucine, norleucine and phenylalanine while in combination with *Beijerinckia* it resulted in the highest amount of threonine, glycine and valine. *Azotobacter* alone increased alanine. Linta[3] (1963) reported an increase in the proportion of protein, nitrogen and amino acids in pea and vetch when inoculated with active strains of rhizobia.

Results of Table I show the synergistic behaviour of *Azotobacter* and the *Rhizobium* strains on crop yield. Similar behaviour of the combined culture is noticeable on the amino acid composition of seeds. By using *Rh.* H_{44} and *Azotobacter* there has been an increase in some amino acids like serine, tyrosine and lysine in comparison with the use of *Rh.* H_{44} or *Azotobacter* alone. Similarly, increases in glycine, valine, tyrosine and histidine were due to *Rh.* H_{45} + *Azotobacter* as compared with the individual cultures. Decrease in aspartic acid and glutamic acid due to *Rh.* H_{44} + *Azotobacter* or *Rh.* H_{45} + *Azotobacter* appears to be more significant in this context because these acidic amino acids are formed first in the process of symbiotic N-fixation (Bala Ravi[1], 1975). Decrease in their amount suggests indirectly their conversion into other amino acids. From the discussion it appears that *Azotobacter* when used along with *Rhizobium*, besides causing the formation of fewer and healthier nodules, helps in the better utilization of symbiotically fixed nitrogen. The other possible beneficial effects of *Azotobacter* in combination with *Rhizobium* have been discussed by Rawat and Sanoria[6] (1976).

TABLE II.
Amino acids expressed in g/100 g protein from seeds of different treatments

Amino acid	Control	*Rhizobium* sp. H_{44}	*Rhizobium* sp. H_{45}	*Beijerinckia indica* J_3	*Azotobacter chroococcum* B_4	*Rh.* sp. H_{44} +*Beij. indica* J_3	*Rh.* sp. H_{44}+*A. chroococcum* B^4	*Rh.* sp. H_{45} +*Beij. indica* J_3	*Rh.* sp. H_{45}+*A. chroococcum* B_4
Aspartic acid	9·69	10·32	11·99*	9·13	10·73	10·89	9·89	10·83	10·70
Glutamic acid	12·71	13·64	14·53*	12·93	14·01	13·93	12·31	13·81	13·79
Hydroxy proline	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Serine	4·74*	3·23	3·45	4·36	3·87	3·56	4·52	3·50	3·40
Threonine	2·49	2·60	2·29	2·53	3·14	3·20	2·79	3·41*	3·17
Proline	4·61*	4·45	3·88	4·32	4·03	3·97	4·53	3·99	4·00
Alanine	3·31	3·35	3·97	3·34	4·30*	4·11	4·01	4·07	4·21
Glycine	5·72	6·67	5·30	5·80	7·21	7·59	6·33	8·01*	7·70
Valine	4·32	4·48	4·53	4·28	5·13	6·09	4·89	6·71*	6·21
Cystine	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Methionine	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ	NQ
Isoleucine	3·78	3·62	4·01*	3·65	3·33	3·62	3·69	3·67	3·71
Leucine	6·95*	6·83	6·79	5·98	6·91	6·73	6·82	6·81	6·81
Norleucine	2·51	2·49	2·53*	2·50	2·51	2·53*	2·50	2·50	2·51
Tyrosine	2·29	2·13	2·04	2·39	2·61	3·92	3·97	3·99	4·01*
Phenylalanine	4·87	3·90	5·64*	4·63	5·07	5·53	5·03	5·20	5·61
Lysine	6·21*	5·01	5·65	6·00	5·16	5·27	5·81	5·18	5·30
Histidine	1·31	1·27	1·09	1·47	2·27	2·32	1·92	2·41	2·71
Ammonia	0·63	0·27	0·52	0·59	0·62	0·67	0·82	0·59	0·62
Tryptophan	ND	ND	ND	ND	ND	ND	ND	ND	ND
Arginine	12·67*	7·87	7·59	10·89	8·13	7·89	7·93	7·50	7·77
TOTAL	88·81	82·13	85·80	84·79	90·03	91·82	87·76	92·18	92·23

Results are expressed:
1. On moisture free basis.
2. Protein conversion factor is used 6·25.

NQ. Not quantitated in acid hydrolysate.
ND. Not detected in acid hydrolysate.
* Maximum among all the treatments.

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